Sylvie Dufour[1], Karine Rousseau[1], Miskal Sbaihi[1], Nadine Le Belle[1], Bernadette Vidal[1], Jacques Marchelidon[1] and Monika Schmitz[2]

[1]Laboratoire de Physiologie Génévale et Compar, Muséum National d'Histoire Naturelle, CNRS, 7, rue Cuvier, 75231 Paris Cedex 05, France.

[2]Department of Aquaculture,Swedish University of Agriculture, Umea, Sweden.

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Multiple Neuroendocrine Regulation of Growth Hormone in the European Eel *Anguilla anguilla*

Abstract

Growth hormone (GH) is involved in the control of various physiological functions in teleosts, including not only body growth but also reproduction, osmoregulation, metabolism and immunity. Most data on GH regulation come from investigations in mammals and studies in teleosts suggest important species-related variations. We developed specific tools to investigate GH regulation in the European eel: purification of pituitary GH, obtention of specific antibodies and development of homologous radioimmunoassay (RIA), cloning of pituitary GH cDNA and obtention of a specific probe for messenger RNA assays, development of an eel pituitary cell culture system for short- and long-term studies of GH synthesis and release.

Investigations on the neuroendocrine control of GH in the eel demonstrated multiple regulations and interactions with other major neuroendocrine axes involved in development, growth, metabolism, and reproduction. Eel somatotrophs exhibit a high autonomous activity. A brain neurohormone,somatostatin (SRIH) exerts a major inhibitory control on GH in the eel as in other teleosts. This inhibitory control has been conserved throughout vertebrate evolution. Insulin-like growth factrors (IGFs), produced by the liver in response to GH, exert a negative feedback on GH synthesis and release, an inhibitory control also strongly conserved among vertebrates. In contrast, GH-releasing factors show large variations among vertebrates and even teleosts. Pituitary adenylate cyclase-activating polypeptide (PACAP), stimulates GH release in the eel as well as in the other teleosts investigated, and may represent an ancestral GH-releasing factor progressively replaced by somatoliberin (GHRH) in tetrapods. Other neurohormones such asgonadoliberins, thyroliberin, dopamine, neuropetide Y, or cholecystokinin, found to exert GH-releasing effects in some but not all teleosts, were inactive on GH release in the eel. In contrast, corticoliberin (CRH) had a strong GH-releasing effect inthe eel, a role possibly related to special developmental and physiological events of the eel biological cycle, such as metamorphosis, fasting, and migration.

Key words: Eel, GH, SRIH, CRH, PACAP

Growth hormone (GH) is a pituitary hormone exerting pleiotropic functions in teleosts as in other vertebrates. Indeed, GH not only plays an essential role in the regulation of body growth and development[1-2], but also participates in metabolic regulations, through effects on glucose utilization[3-4],

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use of fat for energy production[5-8], and protein synthesis[9-11]. GH also influences osmoregulation[12-13], reproduction[14-15], immunity[16-17], and conversion of thyroid hormones[18-19].

In mammals, the regulation of GH release is primarly mediated by the dual actions of two hypothalamic hormones: growth hormone-releasing hormone (GHRH) stimulates, while somatostatin (SRIH) inhibits GH release[20]. In addition, insulin-like growth factor 1 (IGF 1)secreted by the liver under GH control, exerts a negative feedback on GH production[21].

In non - mammalian vertebrates, conservations and variations of factors in volved in the control of GH release have occured. Various hypothalamic neuropeptides, discovered and named according to their hypophysiotropic roles in mammals, have been shown to stimulate GH release in other tetrapods: in birds, for instance, thyroliberin (TRH) is more potent than GHRH to stimulate GH release[22]; in reptiles, corticoliberin (CRH) as well as TRH and GHRH play the roles of GH - releasing factors[23]. The situation appears specially complex in teleosts, in which the factors involved in GH control have been found to be almost as various as the number of species studied. Indeed, GHRH was reported to stimulate GH release in the goldfish[24], rainbow trout[25], tilapia[26],and catfish[27] but had a very low and none dose-dependent effect in the salmon[28]. TRH activated GH release in the goldfish[29] and in the carp[30,31], but not in tilapia[26]. Gonadoliberin (GnRH) strongly stimulated GH release in the goldfish[32], carp [30] and tilapia[26], but not in the trout[34], nor in the catfish[35].

This diversity of GH-releasing factors in teleosts may be related to the pleiotropic functions of GH and the different physiological stages of the fish studied. The variability of the factors controlling GH in teleosts may also reflect the large evolutionary and biological diversity of this group encompassing more than twenty thousands species. We investigated the regulatory factors of GH release in the European eel *Anguilla anguilla* L. Eels are representative of a primitive group of teleosts (Elopomorphs) exhibiting ancestral features of hormonal regulations among vertebrates[36-37]; they undergo an extraordinary life cycle likely controlled by specfic regulatory processes, and represent an important component of natural aquatic ecosystems as well as a major challenge for aquaculture. Getting information on the regulation of GH release in the eel is therefore highly relevant from both fundamental and applied points of view.

Materials and Methods

Fishes

Freshwater yellow and silver eels have been caught from wild populations in pounds of the West and of the North of France. Most of the experimental studies presented here have been performed *in vitro* on juvenile eels at the yellow stage (sedentary, growth phase). Some comparative studies have been performed *in vivo and in vitro*, and at the silver stage (downstream migratory phase).

In vivo studies

Developmental changes in GH levels in natural populations

Developmental changes of pituitary GH content have been investigated in wild populations of European eels during the yellow and silver stage[38-40], and in glass eels collected at their arrival into the estuaries[41].

Experimental changes in GH levels

The effect of experimental fasting was investigated over the long-term (3 months) in yellow female eels[38].

Experimental sexual maturation was induced in female silver eels, according to the procedure of Fontaine et al.[42]: eels received weekly injections, over 4 months, of saline extract of carp pituitary (extract of 1 mg dry carp pituitary /100g body weight/injection). Control eels received saline alone (0.9 % NaCl).

Influence of thyroid hormones on GH production was investigated. Eels received weekly injections of Thyroxine, T4 or tri-iodothyronine, T3 (0.2 mg/ 100g body weight/ injection) suspended in saline or of saline alone (control eels).

Samples

Pituitary was quickly removed after eel decapitation, and kept frozen at -20°C in 0.9 % NaCl until extraction. Pituitaries were extracted by sonication; supernatants obtained after centrifugation were kept frozen until GH radioimmunoassay (RIA). In the case of glass eels, the whole head was submitted to extraction.

Blood was collected in heparinized tubes and plasma samples were kept frozen until GH RIA.

In vitro studies: primary cultures of eel pituitary cells

Cell dispersion

Eels were decapitated and pituitaries quickly removed and placed in ice-cold dispersion buffer: DB, Dulbecco's saline phosphate buffer without Ca2+ and Mg2+, with 100U/m*l* penicillin, 100 µg/m*l* streptomycin and 250 ng/m*l* fungizone (Life Technologies). Dispersion was performed using an enzymatic and mechanical procedure[43]. Pituitary slices (1 mm) were incubated in a solution of 0.8 mg porcine type II trypsin (Sigma)/m*l* DB, at 25° for 1 hr with slow shaking. This trypsin solution was then removed and replaced by a solution of 1 mg soya bean trypsin inhibitor (Sigma) and 1 µg DNAse (Sigma)/m*l* DB for 15 min. Pituitary slices were washed with DB and then mechanically dispersed in DB by repeated passages through a plastic transfer pipette. Cell suspensions were filtered through nylon mesh (30 µm pore size), harvested by centrifugation and resuspended in culture medium(CM): medium 199 with Earle's salts, sodium bicarbonate, 100 U/mlpenicillin, 100 µg/m*l* streptomycin and 250 ng/m*l* fungizone (LifeTechnologies).

Cell culture

Cells were plated on poly-L-lysine (Sigma) precoated plates (Costar), at a density of 62,500 cells/well (96 well plates) for RIA studies or of 500,000 cells/ well (12 well plates) for mRNA studies, in CM. Plates were incubated in a tissue culture incubator (NAPCO), at 18°C under 3% CO_2 and saturated humidity. Cells were allowed to attach and rest for 24 to 72 hours before the start of the experiment (Day 0). Replicates of 6 wells for controls or each treated group were used. Effects of potential inhibitors (SRIH and IGFs) were tested on short-term and long-term experiments up to 12 days of culture. Cultures were stopped before (Day 0) and at the end (Day 12) of the treatments in order to measure GH cell contents (RIA) or GH mRNA levels (dot-blot). Effects of potential stimulators (various neuropeptides, dopamine, or activators of second messenger pathways) on GH release were tested on short-term experiments up to 96 hours. Media and treatments were renewed at each sampling time.

GH intracellular content was extracted by disruption of the cells by an osmotic shock (addition of distilled water) and two repeated cycles of freezing and thawing. Collected media and cell extracts were kept frozen (-20°C) until GH RIA, as previously described[44].

Purification of eel GH

European eel GH has been purified according to the procedure previously described by Marchelidon et al.[45]. Briefly, acetone-dried pituitaries were extracted in 0.9 % NaCl. After centrifugation, the supernatant was passed through Sephacryl S200HR (Pharmacia, Sweden), the fraction containing angGH was further submitted to DEAE-HPLC, then Vydac C4 RP-HPLC chromatography. The N-terminal amino acid sequence and tryptic peptides revealed no protein contamination. The 25 amino acids of the N-terminal sequence of European eel GH were identical to those determined by Yamaguchi et al.[46] for the Japanese eel *Anguilla japonica* GH[38].

Radioimmunoassay (RIA) of eel GH

Production of eel GH antiserum

Purified eel GH (800 μg) was coupled to chicken ovalbumin (1 mg) in presence of glutaraldehyde (2.5 %). Complete Freund adjuvant was added and the emulsified mixture were injected 4 times, at 2-week intervals, to a rabbit. Blood was collected 1 week after the last injection. Antiserum was kept lyophylized, and reconstituted in distilled water before use.

RIA procedure

Purified eel GH was iodinated according to the chloramine T method and separated from free iodine on Sephadex G50 column (Pharmacia)[38].

The RIA was performed in 0.02 M Sodium phosphate buffer, pH 7.4, containing 0.9 % NaCl, 1 % BSA, 0.1 % Tween 20, according to the procedure by Marchelidon et al.[38]. Briefly, eel GH antiserum was used at a dilution of 1:20 000, leading to a maximal binding of 30 % of radiolabelled tracer. Antiserum, tracer, and eel GH standard (purified eel GH) or samples, were incubated for 24 hr at 4°C. Bound fraction was separated by immunoprecipitation (using goat anti-rabbit γ-globulin, Biosys). After centrifugation, the supernatants were decanted and the pellets (bound fractions) were counted in a Kontron γ counter.

Cloning of eel GH cDNA

Cloning of a cDNA fragment encoding European eel GH

A European eel pituitary cDNA library constructed into λ gt10 vector was used to amplify a growth hormone cDNA by polymerase chain reaction (PCR). Two primers were designed based on the nucleotide sequences of the Japanese eel growth hormone cDNA (Primer 1: 5'-GTTAACCGACAGCACCT - 3', Primer 2 : 5'-AGGTCTCCACTTTGTGCATGTC-3')[47]. The PCR products were seperated by 1.5 % agarose gel electrophoresis, the bands of expected size excised, purified with JETquick gel extraction kit (Genomed) and subcloned into pGEM T Easy (Promega). PCR products of three independent PCR amplifications were cloned and sequenced on boths strands using a dye terminator cycle sequencing kit (Amersham Life Science). Nucleotide sequence identity was performed using the BLAST program (Genbank, NCBI).

One major fragment of 481 bp was amplified from the pituitary cDNA ligated into λgt10 vector using Primer 1 and Primer 2. This fragment was cloned and sequenced. Comparison of the nucleotide sequences showed a 98 % nucleotide identity with the Japanese eel growth hormone cDNA[47].

Dot-blot assay of GH mRNA

Cells were scraped from the plates in ice-cold PBS (Life Technologies) collected and total RNA was extracted according to the method previously described[44,48]. Total RNA was blotted on Hybond-N+, Nylon membrane (Amersham) through a Hybri-dot manifold (Life Technologies). The PCR-derived cDNA fragment of eel GH was [32P] dCTP-labeled by random priming with the NEBlot-Kit (New England Biolabs) and used as a probe (overnight hybridization with the membranes at 42°C). After high stringency washing, the membranes were autoradiographed. The membranes were stripped and subsequently hybridized under the same conditions with 32P-labeled eel β-actin cDNA. Scanning densitometry and data processing were performed using a PhosphorImager (Molecular Dynamics).

Results and Discussion

Developmental and physiological changes of GH production

GH is already detectable in the pituitary of glass eels, as shown by immunocytochemistry[38] and by RIA[38,41]. GH immunoreactive cells represent indeed, at this stage, the major cell population of the adenohypophysis[38]. Preliminary data indicate that glass eels undergoing severe physiological stress (small sized, with delayed arrival to the estuaries)

had an elevated pituitary GH content[41]. This suggests that increased GH production may be involved in the mobilization of energy stores necessary for long term survival in stressfull and fasting conditions[2].

Investigations on wild eel populations showed that pituitary GH content steadily increased with body size and age(as determined by otolithometry) during the yellow stage, leading to steady plasma GH levels[39]. This likely reflects the major and steady role played out by GH in the control of body growth throughout the yellow stage.

Submitting yellow eels to long-term (3 months) fasting induced a strong increase in both pituitary GH content and plasma GH levels[38]. This may reflect a the key role exerted by GH, in synergy with cortisol, in the mobilization of metabolic stores for long term survival.

Preliminary analysis of the silvering process indicated a slight increase in pituitary GH content during the first steps of silvering[40]. This stimulation of GH production maybe related to stimulatory roles of GH in the induction of osmoregulatory changes[49], metabolic changes (fasting) and gonadal changes (such as the potentiating effect of GH on hepatic vitellogenesis)[50-51]. In contrast, a decrease in pituitary GH levels was observed at the end of the silvering process, as indicated both by a reduction in the number of GH-immunoreactive cells in the adenohypophysis[38] and the decrease in pituitary GH content, as compared to yelloweels[38-40]. This overall decrease in GH production in silver eels may be related to the cessation of body growth.

Preliminary data indicated a significant increase in pituitary GH mRNA levels in advanced stages of experimentally matured female silver eels. This increase may be related to the involvement of GH in the mobilization of energy and metabolic stores necessary for the dramatic ovarian growth.

Spontaneous release of GH by eel pituitary somatotrophs in vitro

Dispersed eel pituitary cells showed a strong and sustained release of GH in culture media over 12 days in serum-free conditions[52]. These data are in agreement with previous studies performed on organ-cultured whole pituitaries from European eel[53]and Japanese eel[54].

The total amount of GH released over 12 days largely exceeded the initial cell content. This indicated a sustained synthesis of GH by cultured somatotrophs[52].

These results indicate that eel somatotrophs have a high spontaneous activity *in vitro,* and suggest that these cells are submitted to a major inhibitory control *in vivo*[52]. Such a sustained activity of somatotrophs during long-term *in vitro* experiments is also observed in the other teleost species investigated: rainbow trout, *Oncorhynchus mykiss*:[55]; turbot *Psetta maxima*[56]. In the rat, basal release of GH was shown to be related to autonomous electric and ionic properties of somatotrophs, wich exhibit under basal conditions, spontaneous action potentials and Ca^{2+} transients[57]. In the eel, as in other teleosts, the spontaneous release activity of somatotrophs is remarkable.

Inhibitory effect of somatostatin (SRIH) on GH release and synthesis

Somatostatin (SRIH-14) is a neuropeptide discovered and named after its inhibitory action on GH release in mammals. SRIH-14 is expressed in the hypothalamus of all vertebrate classes[58]. SRIH-14 dose-dependently inhibited GH release from eel pituitary cells, with a maximal inhibitory effect of 95 %. Other studies, using short-term (Tilapia[59-60]; trout[61-62]) or long-term (Japanese eel[54]; turbot[56]) experiments, also demonstrated SRIH inhibition of GH basal release in various teleost species. These data indicate that SRIH inhibits basal GH release in all teleosts investigated. In contrast, in other vertebrates, its action may require the presence of stimulators of GH release[52,56].

The inhibitory effect of SRIH was significant at any time of incubation and showed no desensitization over 12days of cell treatment in the eel[52] as well as in the turbot[56]. In contrast, SRIH inhibitory effect was shown to be submitted to rapid desensitization in mammals.

Measurement of GH cell content indicated that SRIH not only inhibited GH release but also GH synthesis in the eel[52], an effect also shown in the turbot[56]. Assay of eel GH mRNA let us to confirm the inhibitory effect of SRIH on GH synthesis by eel somatotrophs *in vitro*. Such an inhibitory effect of SRIH on GH synthesis has not been shown in mammals.

These data indicate that SRIH is a potent inhibitor of GH synthesis and release in the eel as in other teleosts, and may represent the major ancestral control of GH invertebrates.

Negative feedback by liver insulin-like growth factors (IGFs) on GH release and synthesis

As largely studied in mammals, IGF1 is a major growth factor secreted by the liver under the control of GH, and acting in synergy with GH to stimulate body growth. In return, IGF1 exerts an inhibitory feedback control on pituitary GH production[21]. IGF1 has been highly conserved in all vertebrates.

We demonstrated a strong inhibitory effect of IGF1 (up to 85% of inhibition) on GH release by eel pituitary cells.This effect was dose-dependent and not submitted to desensitization. A similar effect was produced by IGF2, while insulin was 1000 less active,indicating the involvement of a IGFtype 1 receptor[52]. The specificity of the inhibitory action of IGFs was further demonstrated by the lack of effect of another growth factor, bFGF (basic fibroblast growth factor). Inhibitory effects of IGFs on GH release by cultured pituitary cells was also demonstrated in short-term studies in the rainbow trout[63] and long-term studies in the turbot[64].

Measurement of GH cell content indicated that IGF1 also strongly inhibited GH synthesis by eel pituitary cells[52], an effect also observed in the turbot[64]. Assay of GH mRNA in eel pituitary cells, confirmed the inhibitory effect of IGF1 on eel GH expression, an action also demonstrated in mammals.

These data suggest that the negative feedback by IGFs may represent an ancestral mechanism of regulation of the somatotropic axis, highly conserved throughout vertebrate evolution.

Lack of GH-releasing effect of GHRHs

Differently from its typical action in mammals, GH-releasing hormone (GHRH) was unable to stimulate GH release in the eel. No effect on eel GH release was observed with human GHRH (Sigma) at any doses and any time of incubation[65-66]. This result was further confirmed by the use of teleost (Goldfish, gf) GHRHs provided by Dr B.K.C. Chow (University of Hong-Kong): carp-like gfGHRH and catfish-like gfGHRH, cloned and synthetized as previously described[67], had no effect on eel GH release[56]. No effects of mammalian nor goldfish GHRHs were neither observed on GH release by turbot somatotrophs[56].

In other teleosts, GHRH was reported to induce a significant increase in GH release in the goldfish[24], rainbow trout[25], tilapia[26] and catfish[27] but had a very limited effect in the salmon[28].

GH-releasing effects of activators of second messenger pathways

In order to assess whether GH release could be stimulated over basal levels in the eel, we investigated the effects of well-known effectors of signal transduction, forskolin (0.1 mM), an activator of adenylate cyclase, and phorbol ester (12-O-tetradecanoylphorbol 13-acetate, TPA) (100 nM), an activator of protein kinase C. We also tested the effect of a depolarizing agent, KCl (50 nM). Each of these three factors induced a significant increase of GH release by eel somatotrophs[56].

Similar results were obtained in some other teleosts (Goldfish[68]; tilapia[60]) in which a stimulation of GH release over basal level could be induced by forskolin and TPA. In contrast, none of these factors induced a significant release of GH by turbot pituitary cells, unless the cells were co-incubated with an inhibitory factor (SRIH)[56]. This suggests that spontaneous basal GH releasere presents the maximal secretory capacity of somatotropes in the turbot but not in the eel or some other teleosts[56].

GH-releasing effect of pituitary adenylate cyclase activating-polypeptide (PACAP)

PACAP was first isolated from the ovine hypothalamus on the basis of its ability to stimulate adenylyl cyclase activity in rat pituitary cells[69]. PACAP is a member of the VIP/secretin/glucagon/GHRH superfamily and its sequence has been highly conserved during evolution from protochordates to mammals[70]. Interestingly, PACAP and GHRH have been shown to be encoded by the same gene in non-mammalian species including teleosts[71-72], amphibians and birds, while they are encoded by two distinct homologous genes in mammals[70].

PACAP was characterized in the eel brain by HPLC and RIA studies. A major form of PACAP immunoreactive material co-eluted with mammalian PACAP 38[66].

Both PACAP 38 and 27 were able to stimulate GH release by eel pituitary cells with the same maximal effect (about 300% over basal release). Dose-response studies indicated that PACAP 38 was approximately 12 times more potent than PACAP 27. In contrast, GHRH and VIP had no significant effect, indicating that the action of PACAP on eel GH release was mediated through a type 1 PACAP receptor. PACAP GH-releasing effect was maximal at 24 h of incubation and then submitted to desensitization.

Immunocytochemical studies in the eel indicated a strong innervation by PACAP-immunoreactive fibers of the pars distalis of the pituitary, where somatotrophs are located[66]. A similar observation was made in the goldfish[73]. In contrast, only rare GHRH immunoreactive fibers were detected in the same region (European eel[74]; goldfish[75]). The difference in expression and localization between PACAP and GHRH maybe due to differential splicing and/or processing of their common precursor[28,71].

PACAP was also shown to be much more effective than GHRH on GH release in the other teleost species so far investigated (Salmon[28]; goldfish[73]; turbot[56]). These data strongly suggest that PACAP, instead of GHRH itself, may play the role of GH-releasing factor in teleosts.

In frog, PACAP and GHRH possess similar stimulatory effects on GH release *in vitro* [76], while in chicken[77] as in mammals, PACAP is a GH-releasing factor but less potent than GHRH[70]. These data suggest that GHRH may have progressively acquired its major role in the stimulatory control of GH during the tetrapod evolution[70].

GH - releasing effect of corticotropin - releasing hormone (CRH)

CRH, is an hypophysiotropic neuropeptide, discovered for its releasing effect on corticotropin (ACTH) in mammals. Its sequence, as well as its ACTH-releasing function, have been highly conserved throughout vertebrate evolution, from teleosts to mammals[78].

We demonstrated that CRH is able to induce GH release by eel somatotrophs in a dose-dependent manner. Its maximal effect (> 400 %) was higher than that of PACAP[65]. As for PACAP effect, GH-releasing effect of CRH was submitted to desensitization, with a maximal effect at 24 h of incubation[65]. In contrast, CRH was not active in another teleost species sofar investigated (Turbot[56]), suggesting that its activity may be related to the special biological cycle of the eel.

Coordinate activation by CRH of the corticotropic and somatotropic axes may underline synergistic controls by cortisol and GH. For instance, GH and

cortisol may interact in osmoregulatory processes for sea-water adaptation in teleosts, by stimulating gill Na, K ATPase and salinity tolerance[13,79]. Moreover, cortisol and GH may also interact in metabolic processes, such as during stress or fasting, by stimulating lipid mobilization in teleosts[6-7] as in mammals[80]. In the eel, mobilization of energy stores is specially required to fullfill gonadal growth and reprod uctive migration[81]. We may raise the hypothesis that CRH may act as a potential coordinator for activating both corticotropic and somatotropic axes during critical developmental or physiological events in the eel, such as silvering, fasting and reproductive migration.

Furthermore, preliminary data indicate that CRH, as well as TRH also stimulates the expression of thyrotropin (TSH) β subunit in our cell culture conditions[82]. CRH also stimulates TSH release *in vitro* in another teleost, the Coho salmon[83].

These data suggest that CRH may play an important role in the activation of various endocrine axes involved in metamorphosis, osmoregulation, and metabolism in the eel and possibly some other teleosts. The GH-releasing role of CRH may have been partially conserved during vertebrate evolution. Indeed, CRH has been shown to induce GH release in Reptiles (turtle) but with a lower potency than TRH or GHRH[23]. In mammals, CRH has no direct effect on GH release by pituitary cells in the rat, but has a stimulatory effect in humans under stress situations (such as fasting or resistance exercise) and in some pathologies (such as depression and acromegaly)[65].

Lack of GH-releasing effect of other neurohormones

Other neuropeptides, shown to induce GH release in some other teleosts, such as gonadotropin-releasing hormone (mGnRH or cGnRH-II: the two native GnRH forms in the eel[84]), thyrotropin- releasing hormone (TRH), neuropeptide Y, cholecystokinin, were inactive in the eel. GnRH was shown to stimulate GH release in the goldfish[32] and tilapia[25], but not in the catfish[27,35] nor in the turbot[56]. TRH, a GH secretagogue *in vitro* in the goldfish[29] and in the carp[30], was also ineffective in tilapia[26] and turbot[56].

Dopamine, active as a GH-releasing factor in goldfish via D1 receptors[85], had no effect on eel GH release[86]. Accordingly, none of the four D1receptor subtypes cloned in the European eel were found to be expressed in the somatotroph area of the eel pituitary[87].

The lack of effect of GnRHs and TRH may be related to teleost species differences, and/or to the physiological stage tested. Indeed, in the goldfish, GH response to GnRH and TRH was higher in sexual mature fish and was potentiated by steroid treatment[29]. Our study which focused on juvenile fish does not exclude that such controls may occur in sexually mature adults.

Inhibitory control of eel GH release and synthesis by thyroid hormones (TH)

Thyroid hormones (TH) and growth hormone (GH) are thought to play synergistic roles in the control of growth and developmental processes invertebrates. Related to this synergism, several physiological crosslinks have been demonstrated between thyrotropic and somatotropic axes. At the central level, CRH was demonstrated to stimulate not only corticotropin release but also to act as a GH-releasing factor and TSH-releasing factor in the eel (see above). At the peripheral level, GH has been reported to increase circulating T3 levels by stimulating peripheral 5'-monodeiodination[18].

Both T3 and T4 inhibited GH release by eel somatotrophs *in vitro*, in a dose-dependent manner compatible with their physiological circulating levels, up to a maximal inhibition of 50 %[88]. Other hormones also acting via the nuclear receptor superfamily, such as sexual steroids (testosterone, estradiol and progesterone) and corticosteroid (cortisol), had no effect on GH

release *in vitro*, underlining the specificity of TH regulatory effect on GH[48,88].The inhibitory effet of TH is in agreement with the previous findings by Baker and Ingleton[53]. Measurement of both GH release and cellular content indicated that TH not only inhibited GH release but also GH synthesis by eel somaptotrophs. Dot blot assay of GH mRNA demonstrated that T3 inhibitory effect on GH synthesis was mediated by a decrease in GH mRNA steady levels.

In vivo administration of triiodothyronine (T3) or thyroxine (T4) also significantly reduced pituitary and serum GH levels in the eel, as measured by homologous RIA, assessing the physiological relevance of the inhibitory effect of TH[88]. The inhibitory control by TH on GH may be viewed as a negative feedback, according to the multiple interactions between somatotropic and thyrotropic axes. TH inhibition of GH may also participate in the reduction of GH production and cessation of body growth at silvering[38-39].

The present study in the eel differs from the few data in other teleost species, which indicated a stimulatory or no effect of TH on GH. In the carp, T_3 increased GHmRNA levels in pituitary fragments *in vitro*[89]. In the rainbow trout, one-weekT_3 treatment *in vivo* increased steady levels of GH mRNA, but produced no alterations in pituitary nor plasma GH levels[90]. *In vitro*, T3 at physiological concentrations, had no significant effect on GH release in tilapia and carp[91-92]. Species-related variations in T_3 effects on GH mRNA levels were also observed among mammals. In the rat, T_3 was shown to stimulate GH mRNA levels in cultured pituitary tumor cell lines[93]. In contrast, T_3 reduced GH mRNA levels in bovine pituitary cells *in vitro*[94], a situation similar to that found for human GH[95].

In contrast, the negative effect of TH on GH in the eel recalls that previously well demonstrated in birds[96, 97], and reptiles[98]. Variations in the control by TH of GH production have occurred among vertebrate species. The negative regulation demonstrated here in an early vertebrate and also found in birds, reptiles and some mammals including human (but not in the rat), may represent an ancestral pattern of GH regulation by thyroid hormones.

Conclusion

Investigation of the neuroendocrine regulation of GH in the European eel shows multiple regulations as well as interactions with other major neuroendocrine axes (thyrotropic and corticotropic) involved in development, growth and metabolism (Figure). Additional interactions may also occur with other neuroendocrine axes, such as the gonadotropic axis, which have not been adressed in the present paper.

Somatotrophs exhibit a high autonomous activity, in the eel as in other teleosts, which may represent an ancestral property of GH basal secretion in vertebrates. Accordingly, the major ancestral control of GH production may be inhibitory, played out by SRIH, and largely conserved throughout vertebrate evolution. The negative feedback by liver IGF may also represent an early emerged and strongly conserved inhibitory control of GH in vertebrates.

The other controls demonstrated in the eel exhibit important variations among vertebrates and even teleosts. PACAP, active in the eel as well as in the other teleosts investigated, may represent an ancestral GH-releasing factor in vertebrates, progressively replaced by GHRH in tetrapods. GH-releasing activity of CRH may be, in contrast, related to the special developmental and physiological events of the eel biological cycle, such as metamorphosis, silvering, fasting, and reproductive migration.

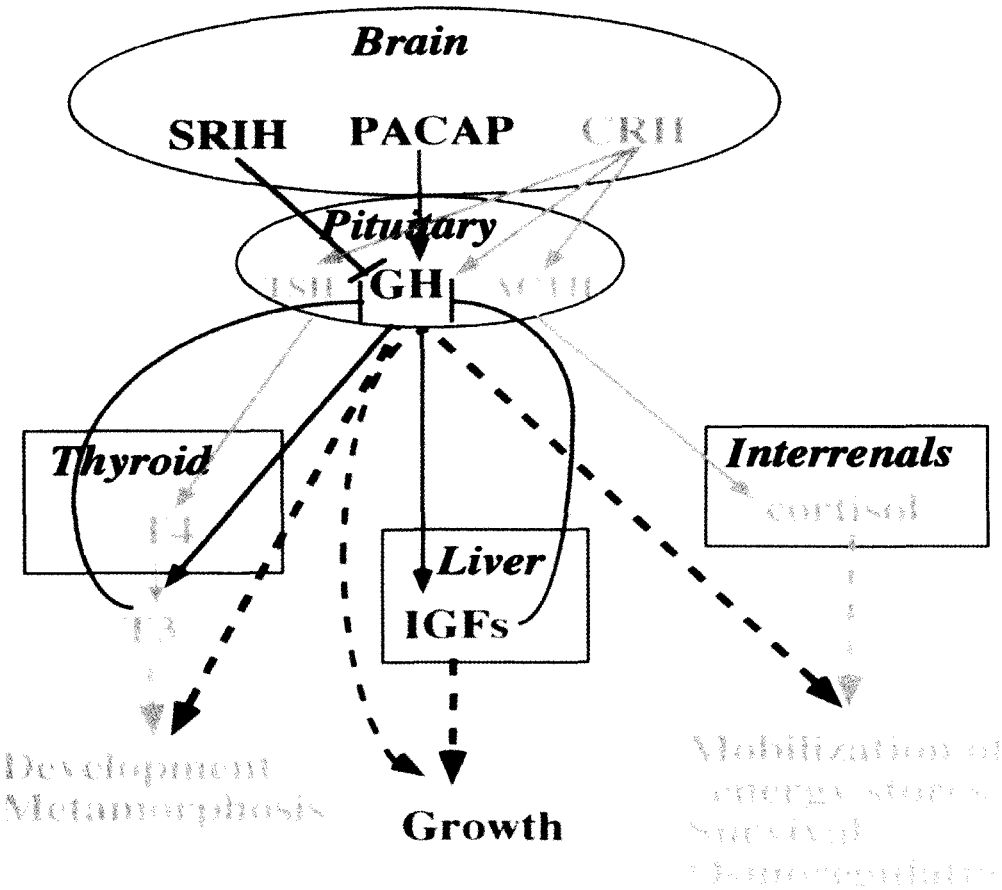

Fig. 1. Multiple neuroendocrine regulation of GH secretion in the European eel:
Eel somatrophs have a high spontaneous activity. GH release is inhibited by somatostatin (SRIH) and stimulated by pituitary adenylate-cyclase activating polypeptide (PACAP) and corticoliberin (CRH). Other neurohormones such as somatoliberin (GHRH), gonadoliberins (GnRH), thyroliberin (TRH), dopamine, neuropeptide Y and cholecystokinin, have no significant effect. PACAP may represent an ancestral GH-releasing factor invertebrates, progressively replaced by GHRH in tetrapods. CRH may beinvolved in the simultaneous activation of several endocrine axes, during specific steps of the eel biological cycle,such as metamorphosis, fasting, reproductive migration. IGFs, produced bythe liver under GH control, exert a negative feedback on GH. Thyroid hormones, the conversion of which is stimulated by GH, also exert anegative control on GH.

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